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       TARALP, ALPAY
       SAYERS, SEHRA

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       PRODUCTS, METHODS OF PREPARATION AND IMMOBILISATION OF PROTEINS
       ON SUPPORT

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<141> 2005-09-20

<150> PCT/TR2003/000019
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                                                     Lys Met Ser Lys
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gga gaa gaa ctt ttc act gga gtt gtc cca att ctt gtt gaa tta gat    345
Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp
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ggt gat gtt aat ggg cac aaa ttt tct gtc agt gga gag ggt gaa ggt    393
Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly
             25                  30                  35
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Page 8

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Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly
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aaa cta cct gtt cca tgg cca aca ctt gtc act act ttc tct tat ggt      489
Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe Ser Tyr Gly
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gtt caa tgc ttt tcc cgt tat ccg gat cat atg aaa cgg cat gac ttt      537
Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg His Asp Phe
    70              75              80

ttc aag agt gcc atg ccc gaa ggt tat gta cag gaa cgc act ata tct      585
Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Ser
85              90              95              100

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Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu
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ggt gat acc ctt gtt aat cgt atc gag tta aaa ggt att gat ttt aaa      681
Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys
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gaa gat gga aac att ctc gga cac aaa ctc gag tac aac tat aac tca      729
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser
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cac aat gta tac atc acg gca gac aaa caa aag aat gga atc aaa gct      777
His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala
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Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala
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gac cat tat caa caa aat act cca att ggc gat ggc cct gtc ctt tta      873
Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu
            185             190             195

cca gac aac cat tac ctg tcg aca caa tct gcc ctt tcg aaa gat ccc      921
Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro
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Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala
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Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys         Ile Pro
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Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
            35   40   45

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
        50   55   60

Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65   70   75   80

Arg His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
                85   90   95

Arg Thr Ile Ser Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
                100   105   110

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
            115   120   125

Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
        130   135   140

Asn Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn
145   150   155   160

Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
                165   170   175

Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
                180   185   190

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Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
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Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
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Thr Thr Glu Asn Leu Tyr Phe Gln Gly Ala Met Gly Gly Thr Val Pro
            20                  25                  30


gta gaa aaa atg agt aaa gga gaa gaa ctt ttc act gga gtt gtc cca       144
Val Glu Lys Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
        35                  40                  45


att ctt gtt gaa tta gat ggt gat gtt aat ggg cac aaa ttt tct gtc       192
Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val
    50                  55                  60


agt gga gag ggt gaa ggt gat gca aca tac gga aaa ctt acc ctt aaa       240
Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
65                  70                  75                  80


ttt att tgc act act gga aaa cta cct gtt cca tgg cca aca ctt gtc       288
Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
                85                  90                  95


act act ttc tct tat ggt gtt caa tgc ttt tcc cgt tat ccg gat cat       336
Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
                100                 105                 110


atg aaa cgg cat gac ttt ttc aag agt gcc atg ccc gaa ggt tat gta       384
Met Lys Arg His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val
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Gln Glu Arg Thr Ile Ser Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg
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gct gaa gtc aag ttt gaa ggt gat acc ctt gtt aat cgt atc gag tta    480
Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu
145             150             155             160

aaa ggt att gat ttt aaa gaa gat gga aac att ctc gga cac aaa ctc    528
Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu
                165             170                 175

gag tac aac tat aac tca cac aat gta tac atc acg gca gac aaa caa    576
Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln
                180             185             190

aag aat gga atc aaa gct aac ttc aaa att cgc cac aac att gaa gat    624
Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp
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gga tcc gtt caa cta gca gac cat tat caa caa aat act cca att ggc    672
Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
    210             215             220

gat ggc cct gtc ctt tta cca gac aac cat tac ctg tcg aca caa tct    720
Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
225             230             235             240

gcc ctt tcg aaa gat ccc aac gaa aag cgt gac cac atg gtc ctt ctt    768
Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu
                245             250             255

gag ttt gta act gct gct ggg att aca cat ggc atg gat gag ctc cgt    816
Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Arg
            260             265             270

cga caa gct tgc ggc cgc act cga gca cca cca cca cca ctg aga       864
Arg Gln Ala Cys Gly Arg Thr Arg Ala Pro Pro Pro Pro Leu Arg
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Ser Gly Cys
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            35                  40                  45


Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val
        50                  55                  60


Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
65                  70                  75                  80


Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
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Gln Glu Arg Thr Ile Ser Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg
    130   135   140

Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu
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Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu
            165   170   175

Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln
            180   185   190

Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp
        195   200   205

Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
    210   215   220

Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
225   230   235   240

Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu
            245   250   255

Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Arg
            260   265   270

Arg Gln Ala Cys Gly Arg Thr Arg Ala Pro Pro Pro Pro Leu Arg
            275   280   285

Ser Gly Cys
    290

<210>   8
<211>   17
<212>   DNA
<213>   Artificial sequence

<220>
<223>   Frame adapter used for prevention of frameshift mutation as a
        result of plasmid modification

<220>

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<221>   misc_feature
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<223>   Frame adapter used for prevention of frameshift mutation as a

        result of plasmid modification

<400>   8
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<210>   9
<211>   5
<212>   PRT
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<220>
<223>   Peptide design based on size and flexibility to act as a linker
        between the tag and GFP protein segments


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<221>   MISC_FEATURE
<223>   Peptide design based on size and flexibility to act as a linker
        between the tag and GFP protein segments

<400>   9

Met Gly Gly Thr Val
1               5


<210>   10
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<213>   Artificial sequence

<220>
<223>   Peptide design based on charge and shape to bind the

        expressed protein to a suitably interactive surface


<220>
<221>   MISC_FEATURE
<223>   Peptide design based on charge and shape to bind the

        expressed protein to a suitably interactive surface

<400>   10

His His His His His His
1               5


<210>   11
<211>   238
<212>   PRT
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<220>
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<223>   Green fluorescent peptide coded by pGFPuv plasmid, permitting
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easy visualisation and quantification based on fluorescence
properties

<400> 11

Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
1   5   10   15

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
            20   25   30

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
            35   40   45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
        50   55   60

Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg
65   70   75   80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
                85   90   95

Thr Ile Ser Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
            100   105   110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
        115   120   125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
    130   135   140

Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn Gly
145   150   155   160

Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
                165   170   175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
            180   185   190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
        195   200   205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
    210   215   220

Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225   230   235

<210> 12
<211> 291
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<213> Artificial sequence

<220>
<223> Completed peptide based on Hisx6 tag, short physically flexible
      linker and green fluorescent protein coded by pETM-GFP-Imm to
      impart fluorescence properties, allowing easy immobilisation with
      retention of bioactivity, visualisation and quantification

<220>
<221> MISC_FEATURE
<223> Completed peptide based on Hisx6 tag, short physically flexible
      linker and green fluorescent protein coded by pETM-GFP-Imm to
      impart fluorescence properties, allowing easy immobilisation with
      retention of bioactivity, visualisation and quantification

<400> 12

Met Lys His His His His His Pro Met Ser Asp Tyr Asp Ile Pro
1   5   10   15

Thr Thr Glu Asn Leu Tyr Phe Gln Gly Ala Met Gly Gly Thr Val Pro
            20   25   30

Val Glu Lys Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
        35   40   45

Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val
    50   55   60

Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
65   70   75   80

Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
            85   90   95

Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
            100   105   110

Met Lys Arg His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val
        115   120   125

Gln Glu Arg Thr Ile Ser Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg
    130   135   140

Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu
145   150   155   160

```
Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu
                165             170             175

Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln
                180             185             190

Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp
            195             200             205

Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
        210             215             220

Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
225             230             235             240

Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu
                245             250             255

Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Arg
                260             265             270

Arg Gln Ala Cys Gly Arg Thr Arg Ala Pro Pro Pro Pro Leu Arg
            275             280             285

Ser Gly Cys
        290
```

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<210>   13
<211>   29
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<220>
<223>   Frame adapter

<400>   13

gtacgccatg ggaggcacgg taccttgtg                                    29
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